universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Effects of dietary fatty acids on the course of gestation and lactation in guinea pigs“

verfasst von / submitted by

Birgit Klinc, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2017 / Vienna 2017

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 878
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Verhaltens-, Neuro- und Kognitionsbiologie
Betreut von / Supervisor:	ao. Univ.-Prof. Dr. Eva Millesi

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisor Eva Millesi for her guidance and useful feedback during this project. Without her support, this thesis would not have been possible.

Special thanks to my co-supervisor Matthias Nemeth for his remarks and encouragement through the learning process of this master thesis. I could not imagine having a better co-supervisor, with a unique knowledge of guinea pigs, fatty acids, statistics and so many other things and always willing to help me throughout any kind of issues.

Many thanks to my colleague, Bettina Schragner, who was a reliable team partner during the whole project and for helping me collecting the data and feeding the guinea pigs. Furthermore, I am very thankful for the support of Elisabeth Pschernig for helping me analyzing the samples and extending my skills in the laboratory.

Especially I would like to express my gratitude to my parents for giving me the possibility to study biology and always providing me with their support and love.

Finally, I would like to thank my partner, my sister and my friends for all their help, love and continuous encouragement throughout my years of study and through the process of researching and writing this thesis.

This accomplishment would not have been possible without them.

Thank you.

TABLE OF CONTENTS

INTRODUCTION

Nutrition plays an important role in life, especially during energy-demanding periods such as pregnancy and lactation. Fat is the major energy source with 1 g providing 9 kcal (Lunn et al. 2006). The properties of fatty acids are determined by the length of the carbon chain and the number of double bonds. Based on this, fatty acids can be classified as saturated fatty acids (SFAs), without double bonds and unsaturated fatty acids (UFAs) containing double bonds. UFAs have different properties depending on the position of the first double bond in relation to the methyl end (Mattos et al. 2000). Polyunsaturated fatty acids (PUFAs), in which the double bond is located three carbons from the methyl end, are known as omega-3, six carbons from the methyl end as omega-6 and nine carbons from the methyl end as omega-9 PUFAs (Wathes et al. 2007). Due to a lack of appropriate desaturase enzymes, the omega-3 α-linolenic acid (ALA) and omega-6 linoleic acid (LA) have to be ingested by the diet (Yehuda et al. 1999; Wathes et al. 2007). Correspondingly, these fatty acids are essential for most mammals (essential fatty acids, EFAs), because they cannot be synthesized de novo (Wathes et al. 2007).

Polyunsaturated fatty acids are an important component of the cell membrane (Klausner et al. 1980) and they are able to influence various functions, such as the cell membrane fluidity (Stillwell et al. 1993), properties of transporters (Youdim et al. 2000), ion channels, neurotransmitters (Yehuda et al. 2005), receptor formation and enzyme activity (Yehuda et al. 1997; Yehuda et al. 2005).

PUFAs therefore have been shown to positively affect cognitive functions, stress-related anxiety (Ferraz et al. 2011) and aggressive behavior (Fedorova & Salem 2006). Learning and memory can also be improved by these fatty acids, via increased the learning capacity and learning abilities (Bourre et al. 1989; Yamamoto et al. 1988). In guinea pigs, such effects differed between the sexes (Nemeth et al. 2015). While females fed with omega-3 and omega-9 fatty acids showed an improvement in spatial learning abilities, no influence of the diet was found in males (Nemeth et al. 2015).

Furthermore beneficial effects of PUFAs on several brain-mediated diseases have been reported, like positive impacts on schizophrenia (Fenton et al. 2000; Du Bois et al. 2005), hyperactivity (Sinn et al. 2008), mood disorders (Buydens-Branchey et al. 2008), depression (Colangelo et al. 2009) and Alzheimer disease (Kalmijn 2000; Morris et al. 2003a). Additionally PUFAs had positive effects on cardiovascular diseases, Type 2 diabetes (Wang et al. 2006;

Nettleton & Katz 2005) and replacement of saturated fatty acids with unsaturated fatty acids lowered total plasma cholesterol and LDL cholesterol (Abbey et al. 1994).

Regarding stress response, unsaturated fatty acids can affect the hypothalamic–pituitary–adrenal axis (HPA axis) and reduce cortisol concentrations (Ferraz et al. 2011). The secretion of glucocorticoids from the adrenal cortex, usually in response to a stressor, is stimulated by adrenocorticotropin (ACTH) from the anterior pituitary, caused by the release of corticotropin releasing hormone (CRH) from the hypothalamus (Sapolsky et al. 2000).

In contrast to PUFAs, saturated fatty acids have been reported to be positively correlated with dementia (Kalmijn et al. 1997), Alzheimer (Morris et al. 2003b) and cardiovascular diseases (Pietinen et al. 1997).

Considering behavioral aspects, results are contradictory. In mice, the saturated fatty acid palmitic acid induced anxiety-like behavior (Moon et al. 2014) however, in rats myristic acid produced anxiolytic effects (Contreras et al. 2014). Nevertheless, feeding guinea pigs with saturated fatty acids lowered their frequencies of aggressive behavior (Nemeth et al. 2016a).

Fatty acids can be obtained from different sources. Unsaturated fatty acids can be found in fatty fish, cod liver, fish oil, soya bean oil, rape seed oil or linseed oils, whereas saturated fatty acids are contained in milk and meat products or coconut fat (Rustan & Drevon 2005).

During the energy demanding gestation period appropriate nutrition is highly relevant for the development of the fetus, particularly efficiently energy delivering fatty acids. N-3 polyunsaturated fatty acids during gestation and lactation can improve growth, cognitive functions, visual acuity and neurological development of the offspring (Lunn & Theobald 2006; Campoy et al. 2012). In women, 300 mg/d of the omega-3 DHA (Docosahexaenoic acid) during pregnancy and lactation is generally recommended (Simpoulos et al. 1999). Nemeth and colleagues (2017), showed that a diet high in polyunsaturated fatty acids during pregnancy resulted in improved gestational effort, determined by a higher body mass gain during pregnancy. In contrast, a diet high in saturated fatty acids led to lower body mass gain and smaller litter sizes (Nemeth et al. 2017).

The domestic guinea pig (*Cavia aperea f. porcellus*) is an ideal model species to study the effects of dietary fatty acids during gestation and lactation, because of its relatively long gestation phase (68 days, Rowlands 1949). The offspring is extremely precocial after birth, highly developed, independent and mobile (Künkele & Trillmich 1997). This results in high metabolic maintenance costs for the pups in the first days of their lives, in contrast to the lower

and slowly increasing costs for more dependent and inactive altricial newborns (Künkele & Trillmich 1997). Furthermore, this species is well studied with regard to their physiological and behavioral response in different situations, like social behavior during group-housing, stress response and juvenile development (Rood 1972; Künzl & Sachser 1999; Machatschke et al. 2004; Nemeth et al. 2016b; Bauer et al. 2008;).

In this study pregnant domestic guinea pigs were supplemented with either walnut oil (high in unsaturated fatty acids) or coconut fat (high in saturated fatty acids) to determine potential effects of both fatty acid types. Based on the importance of energy intake during gestation and lactation and the reported opposite effects of saturated and unsaturated fatty acids, we focused on the following aspects: reproductive success, reproductive phases, behavior and juvenile development.

MATERIAL & METHODS

Ethical statement

All husbandry and experimental procedures were performed in line with EU Directive 2010/63/EU and the Austrian laws for animal experiments and animal keeping. The study was approved by the internal board on animal ethics and experimentation of the Faculty of Life Science of the University of Vienna (# 2014-005) and the Austrian Federal Ministry of Science and Research (BMWF-66.006/0024-II/3b/2013).

Animals

Sixteen pregnant domestic guinea pigs (*Cavia aperea f. porcellus*) were used for this study (control group: 7 females, SFA group: 7 females, UFA group: 2 females). Despite several mating attempts in each group, only two UFA females got pregnant and thus had to be excluded from further comparisons between the groups due to the low sample size. Individual age did not differ between the three groups (mean age 603.69 ± 42.6 days; $\chi^2 = 1.934$, $p = 0.38$). All animals were bred at the Department of Behavioral Biology, University of Vienna and were accustomed to the daily contact with humans. Furthermore, the guinea pigs, which participated in this study, originated from the F0 generation of a long-term study on effects of dietary fatty acids. Both parents were fed with either SFA or UFA – enriched diets respectively or were in the control group. Thus the F1 generation was already confronted with the respective diet prenatally.

Due to natural fur markings, individuals could be easily identified. None of the animals had been pregnant before this study. Guinea pigs are pregnant for about 68 days (Rowlands 1949) and have an estrous cycle of around 16 days (Selle 1922; Joshi et al. 1973). The estrus correlates well with the opening of the vaginal membrane (Young 1937), which was inspected visually daily during mating. Therefore, the day of conception and gestation duration could be easily determined.

Housing Conditions

Animal enclosures (3.2 m^2 for each group) were environmentally enriched with shelters and platforms and the floor was covered with standard woodchip bedding material, which was renewed at least once a week. Animals were housed on a light-dark cycle 12/12 h (lights on at 7:00 a. m.), at 20 ± 2°C and a humidity of 50 ± 5 %.

Animals were assigned to three feeding groups (based on the diet of their parents): control group received a standard diet for guinea pigs (ssniff V2233, ssniff Spezialdiäten GmbH, Soest, Germany), UFA group received ssniff pellets supplemented with 10% walnut oil (high in PUFAs) and SFA group received ssniff pellets supplemented with 10 % coconut fat (high in SFAs). For a detailed composition, see Table 1. Each group was provided with 50 g hay and water ad libitum in several drinking bottles.

Table 1. Fatty acid composition of walnut oil and coconut fat.

Values in g/100g, based on US Department of Agriculture and National Nutritient Database.

Fatty acid	walnut oil	coconut fat
C 4:0	/	0.06
C 6:0	/	0.75
C 8:0	/	7.89
C 10:0	/	5.60
C 12:0	/	44.48
C 14:0	/	16.66
C 16:0	4.40	8.18
C 18:0	1.67	2.84
C 20:0	0.06	0.04
Total SFAs	**6.13**	**86.50**
C 18:01	8.80	5.68
C 20:01	0.13	/
Total MUFAs	**8.93**	**5.68**
C 18:02	38.09	1.45
C 18:03	9.08	/
Total PUFAs	**47.17**	**1.45**

Experimental design & sample collection

Animals were weighed 2-3 times a week on a standard kitchen balance (accuracy ± 1 g) at 9:30 a.m. Group-housing videos were done in 3-week intervals at 9:00 a.m. for 30 minutes. During these 30 minutes, all shelters were removed and the groups were video recorded with cameras fixed on the ceiling and located above the enclosures.

Openfield videos and saliva/feces sampling were also done every 3 weeks at 10:00 a.m. Each individual was separated from the group, followed by inserting a standard cotton bud into animal's cheek pouch for about 30 seconds to collect saliva samples and to determine the standard cortisol values. Afterwards they were put into the openfield area and video recorded

with a camera fixed on the ceiling. After 10 minutes, saliva was collected again and the animal was relocated in its group enclosure. Feces from the openfield area were collected to analyze progesterone/estradiol concentrations. Urine contaminated feces were excluded and the soiled bedding material renewed. Saliva samples were centrifuged (10000 rpm, 5 minutes) and the brackets and the dry cotton buds were removed. Afterwards saliva and fecal samples were stored at -20 °C until analysis. To avoid diurnal fluctuations of the hormone levels, sampling was carried out at the same time of day (Sachser 1994).

Hormone analysis

Cortisol, progesterone and estradiol concentrations were analyzed via biotin-streptavidin enzyme-linked immunoassay (Palme & Möstl 1993; Palme & Möstl 1997). Enzymes and antibodies were purchased from the University of Veterinary Medicine in Vienna, Austria.

The saliva samples they were diluted 1:50 after defrosting.

Fecal samples were dehumidified at 60 °C, homogenized and diluted with 80 % methanol. After centrifugation (3000 rpm, 15 minutes) fecal samples for progesterone analyses were diluted 1:50 and fecal samples for estradiol analyses were diluted 1:30. All hormones were analyzed with specific antibodies and run in duplicates.

Intraassay and interassay coefficients were as follows: cortisol 11.61 % (intra) and 0.37 % (inter), progesterone 12.44 % (intra) and 9.59 % (inter), estradiol 7.84 % (intra) and 7.66 % (inter).

Behavioral analysis

Videos were filmed with GoPro Hero 3 (resolution: 5 MP, field of view: 170 °) and analyzed with Solomon Coder Version 16.06.26.

For each individual, behavior and lactation performance was measured via continuous/all occurrence sampling method (Altman 1974). Social behavior and grooming was measured in frequencies, whereas movement, “side by side”, time spent in the center of the openfield area and lactation (time females spent lactating) was measured in durations.

For group-housing during gestation and lactation behavioral categories and definitions followed by Rood 1972: 1) movement 2) “side by side“ 3) sociopositive behavior: social grooming, nose-nose, naso-anal, lactation (own/other infants) 4) agonistic behavior: displacement, bite, head-thrust, stand-threat, chase, fight, harassment, kick-back, rumba-rumble, mounting.

During gestation, behavior in the openfield area was defined as followed: 1) movement 2) time spent in the center of the openfield area.

During lactation openfield categories were the same as during gestation, but the categories 3) “side by side” and 4) lactation duration, were added.

Gestation duration was defined as the day of parturition minus the day of conception (first day of the opening of the vaginal membrane during mating).

The gestational effort of the females was calculated as follows: total litter weight (g)/maternal body weight at conception (g) x 100, (Laurien-Kehnen & Trillmich 2004).

Statistical analysis

Before analysis, data was tested for normal distribution via Shapiro-Wilk test and equally variances were examined with the Levene’s test for homogeneity. If normal distribution was not given, nonparametric tests were used.

Statistical calculations were done with SPSS statistics for Windows (IBM Corp., 2011, Version 20.0. Armonk, NY). Due to the small amount of pregnant UFA females, they were excluded from statistical analysis.

Control and SFA group were compared with Students t-test (normal distributed) or the non-parametric Mann-Whitney U test. Comparisons within a group between the different stages (gestation and lactation) were done using either t-tests or non-parametric Wilcoxon tests.

Significance level was set at $p \leq 0.05$.

RESULTS

Reproductive output

Mean litter size of alive and dead born offspring was 3.0 (± 1.41) in control and 3.29 (± 0.75) in SFA females. The UFA females had only two litters, one with three pups, one with five pups. Due to the small sample size the UFA group could not be included in statistical analyses.

Neither the number of alive born pups, nor of dead born ones differed significantly between the control and SFA group (born alive: $F_{0.324} = 0.58$, $p = 0.4$; born dead: $F_{1.507} = 0.243$, $p = 0.825$) (Fig. 1). In both groups the proportion of dead born pups ranged between 30.4 % and 38.1 % (Fig. 1). In the UFA group, however, all pups were born alive.

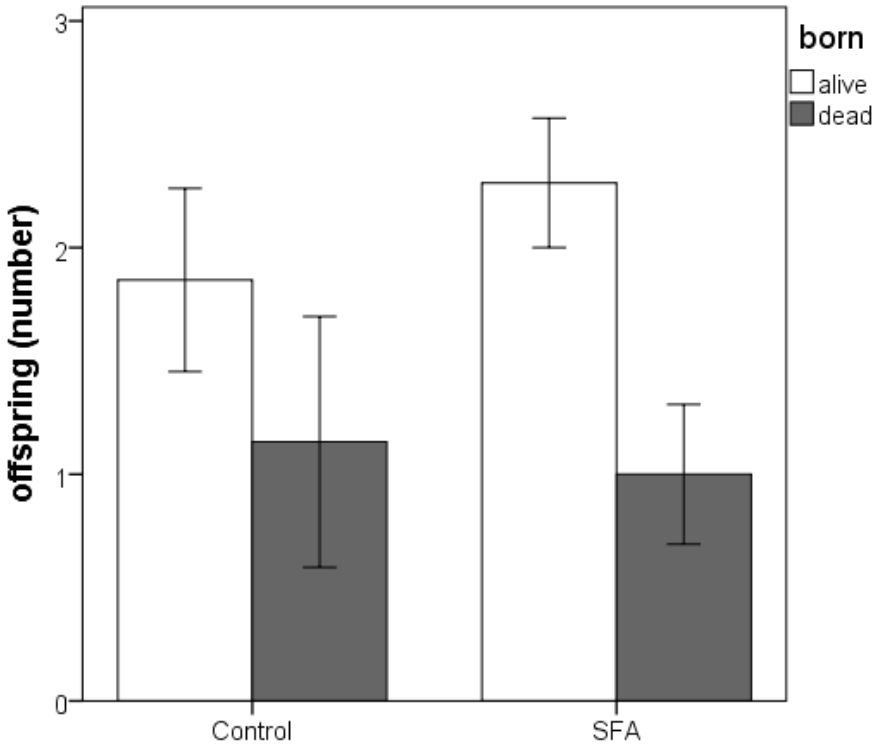

Fig. 1. Offspring number (born dead/alive) of control and SFA females (means ± SE).

The offspring sex ratio did not differ between the groups ($F_{0.007} = 0.937$, $p = 0.432$). Thus, the control and SFA group had similar litter sizes, sex ratio, and an equal ratio of alive/dead born pups (Table 2).

Table 2. Newborn female, male and total pups (total/alive/dead) in the three diet groups.

		Control (7)	SFA (7)	UFA (2)
total	female	9 (42.86 %)	14 (60.87 %)	4 (50 %)
	male	12 (57.14 %)	9 (39.13 %)	4 (50%)
	total	**21**	**23**	**8**
alive	female	6 (46.15 %)	10 (62.50 %)	4 (50 %)
	male	7 (53.85 %)	6 (37.50 %)	4 (50 %)
	total	**13**	**16**	**8**
dead	female	3 (37.50 %)	4 (75 %)	0 (0 %)
	male	5 (62.50 %)	3 (25 %)	0 (0 %)
	total	**8**	**7**	**0**

Body mass at birth of offspring born alive did not differ between the sexes and was similar in both groups (between groups & both sexes: $F_{0.469} = 0.499$, $p = 0.305$ (Fig.2); between groups & males: $F_{1.8} = 0.207$, $p = 0.526$; between groups & females: $F_{0.512} = 0.486$, $p = 0.57$).

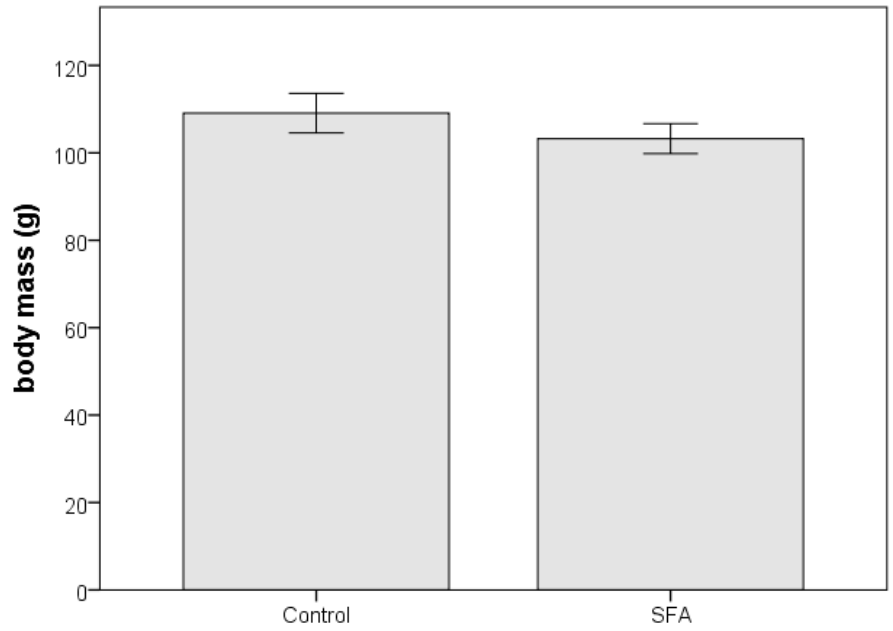

Fig. 2. Body mass at birth (g) in control (n = 13) and SFA (n = 16) offspring (sexes pooled, averaged per litter, means ± SE).

Reproductive phases

Gestation duration and gestational effort did not differ between the control and SFA group (Table 3).

Table 3. Gestation duration (days) and gestational effort (%) in control and SFA females (means ± SE).

Reproductive parameter	p	mean (± SD)
Gestation duration	$F_{2.120} = 0.171$; $p = 0.65$	Control: 68.57 d ± 2.10
		SFA: 69.00 d ± 1.29
Gestational effort	$F_{3.386} = 0.091$, $p = 0.986$	Control: 32.18 % ± 5.24
		SFA: 32.08 % ± 1.70

Body mass both during gestation and lactation was significantly higher in SFA females than in controls (Fig. 3). Furthermore, both groups had lower mass during lactation than during gestation (control: Z = -5.081, p < 0.001; SFA: Z = -5.177, p < 0.001) (Fig. 3).

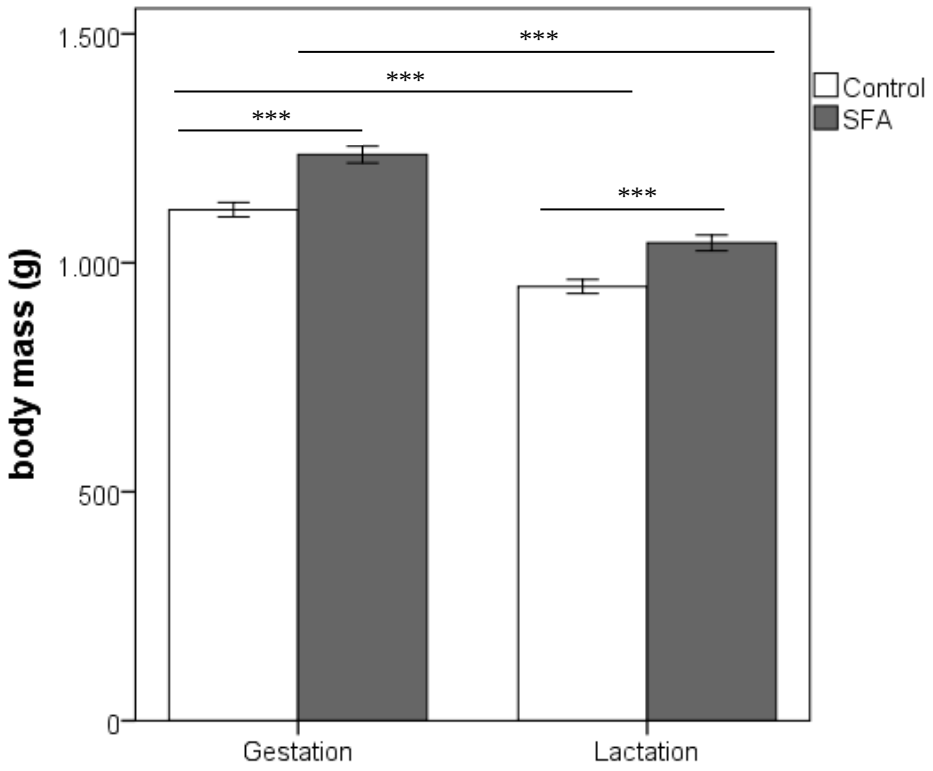

Fig. 3. Body mass (g) during gestation and lactation of control (n = 7) and SFA (n = 7) females (gestation: Z = -5.146, p < 0.001; lactation: Z = -3.373, p = 0.001) (means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

No significant differences in relative body mass changes between control and SFA females during gestation or lactation were detected (gestation: $F_{1.720}$ = 0.214, p = 0.61; lactation: $F_{2.354}$ = 0.151, p = 0.729) (Table 4).

Table 4. Body mass changes(%) during gestation and lactation in control and SFA females (means ± SE).

Stage	Group	Mean (± SD)
Gestation	Control	34.7 (± 17.30)
	SFA	38.4 (± 6.71)
Lactation	Control	-2.4 (± 8.90)
	SFA	-3.7 (± 4.14)

Both groups significantly lost mass after parturition (control: -315.1 g (± 131.4), T = 5.6, p = 0.001; SFA: -370.4 g (± 61.4), T = 15.172, p > 0.001), and mass loss was similar in SFA and control females ($F_{8.607}$ = 0.13, p = 0.9).

During gestation SFA females had higher fecal estradiol metabolite (FEM) concentrations than control females ($F_{1.746}$ = 0.196, p = 0.028) (Fig. 4). Due to the low sample size, comparisons between the control and SFA group during lactation could not be performed.

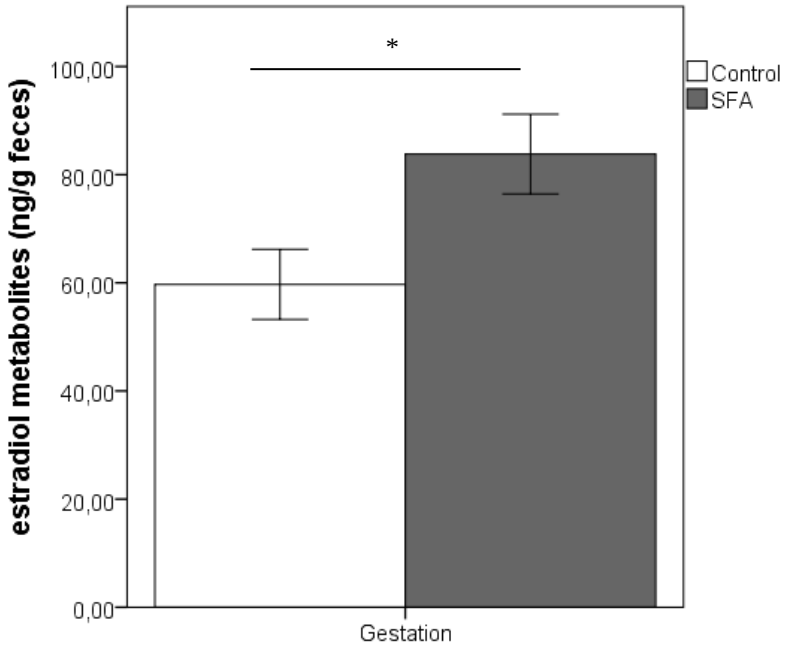

Fig. 4. Estradiol metabolites during gestation in the control (n = 5) and SFA (n = 7) group (means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

SFA females had lower levels of fecal progesterone metabolite (FPM) concentrations during gestation than control females (Z = -2.775, p = 0.006) (Fig. 5). FPM concentrations during lactation could not be compared due to low sample sizes.

Accordingly, SFA females also had a lower progesterone/estradiol ratio during gestation than controls (Z = -3.226, p = 0.001).

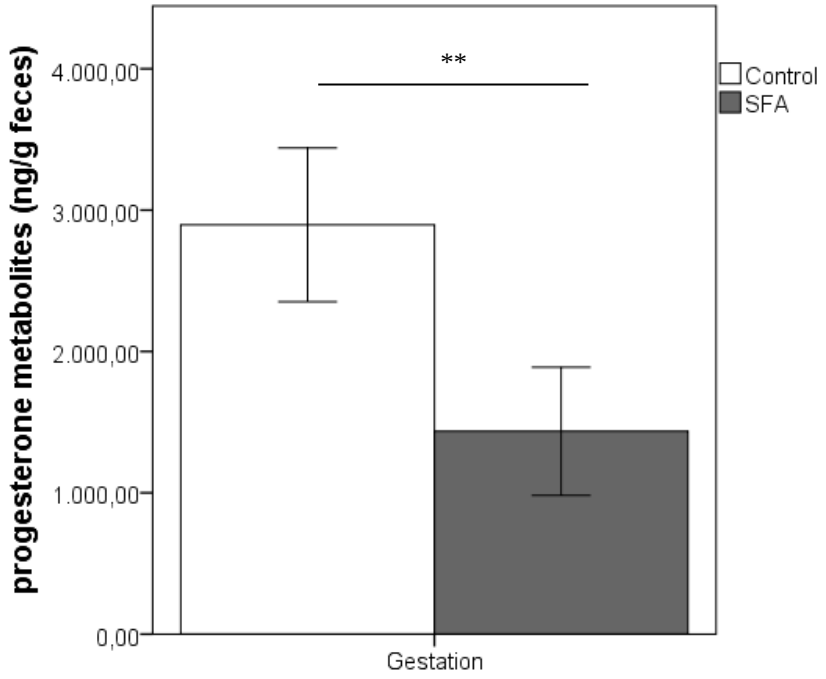

Fig. 5. Progesterone metabolites during gestation in the control (n = 5) and SFA (n = 7) group (means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

Regarding basal saliva cortisol concentrations, no differences between control and SFA group during both reproductive stages were found (gestation: $F_{1.697}$ = 0.201, p = 0.81; lactation: $F_{0.022}$ = 0.886, p = 0.217). Furthermore, basal cortisol values did not differ between gestation and lactation in both groups (control: gestation: 16.02 ± 1.96, lactation: 21.68 ± 4.22, Z = -1.604, p = 0.109; SFA: gestation: 13.35 ± 1.86, lactation: 14.15 ± 2.99, T = 0.089, p = 0.932,).

Behavior

During pregnancy, SFA females showed "side by side" with other females in the group-housing situation more often than the control group (Table 5). Movement duration as well as the frequencies of agonistic and sociopositive behavior did not differ significantly between the diet groups (Table 5).

Table 5. Comparisons of movement, "side by side", agonistic and sociopositive and behavior during group-housing in the course of gestation and lactation between control and SFA females (means ± SE).

Behavior	Stage	p	Mean (± SD)
Movement duration	Gestation	$F_{2.208} = 0.145$, p = 0.799	Control: 67.05 (± 31.57)
			SFA: 70.19 (± 46.33)
	Lactation	$F_{0.001} = 0.976$, p = 0.257	Control: 90.00 (± 54.2)
			SFA: 121.29 (± 40.17)
"Side by side" (with other females)	Gestation	Z = -2.579, **p = 0.01**	Control: 149.00 (± 164.24)
			SFA: 304.67 (± 221.17)
	Lactation	$F_{0.418} = 0.531$, p = 0.753	Control: 178.17 (± 138.01)
			SFA: 206.43 (± 172.15)
Agonistic (with other females)	Gestation	Z = -0.348, p = 0.727	Control: 2.19 (± 2.75)
			SFA: 1.57 (± 1.29)
	Lactation	$F_{12.294} = 0.005$, p = 0.169	Control: 3.83 (± 4.26)
			SFA: 1.00 (± 1.15)
Sociopositive (with other females)	Gestation	Z = -1.432, p = 0.152	Control: 2.95 (± 2.54)
			SFA: 1.90 (± 2.81)
	Lactation	Z = -1.455, p = 0.146	Control: 4.50 (± 1.62)
			SFA: 2.43 (± 1.62)

In the group-housing situation, differences between the reproductive stages were found in the SFA group, in that the females moved more during lactation than during gestation (control: T = -1.576, p = 0.176; SFA: T = -3.958, p = 0.007). Neither in the control, nor in the SFA group differences between the stages could be detected in: "side by side" (control: Z = -0.105, p = 0.917; SFA: T = 1.561, p = 0.169), agonistic behavior (control: T = -1.146, p = 0.304; SFA: Z = -1.577, p = 0.115) and sociopositive behavior with other females (control: T = -1.049, p = 0.342; SFA: Z = -0.677, p = 0.498). Concerning interactions of females with their infants

during group-housing, no differences between the groups could be found in any of the behavioral parameters ($p > 0.05$).

The time females spent lactating the offspring (lactation duration) did not differ between the control and SFA group during group-housing ($Z = -1.077$, $p = 0.282$). However, control females lactated their own infants significantly longer than other pups, whereas in SFA females no difference in the duration of lactating own and other juveniles was found (control: $Z = -2.159$, $p = 0.031$; SFA: $Z = -0.065$, $p = 0.948$) (Fig. 6).

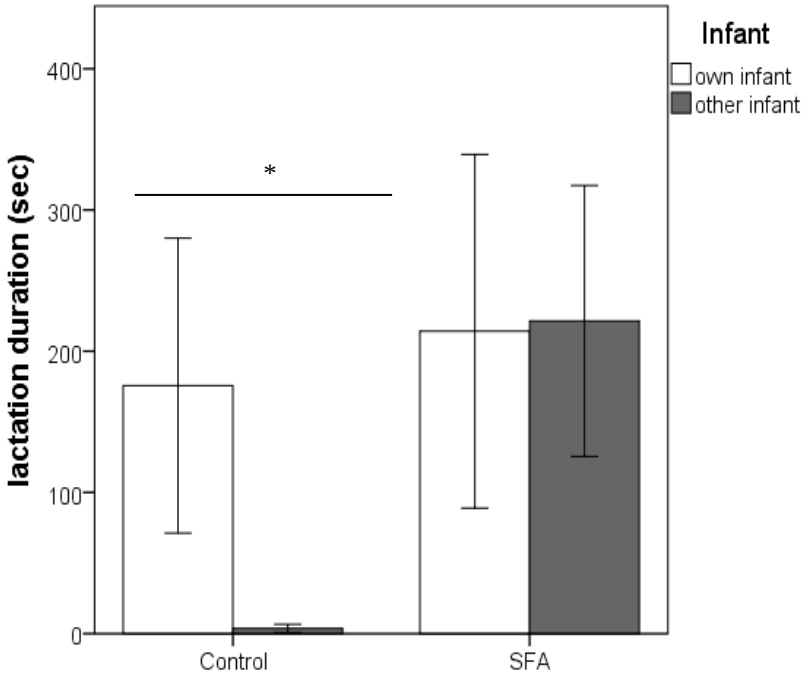

Fig. 6. Lactation duration of own and other infants in the control and SFA group during group-housing (means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

No significant differences were detected regarding the rejection of suckling attempts between the groups or within a group between own and other infants (between control and SFA: $Z = -0.262$, $p = 0.794$; within control group: $Z = -1.878$, $p = 0.06$; within SFA group: $Z = -0.784$, $p = 0.433$).

Openfield test

In the openfield test SFA females moved less than the control group during gestation, while no other differences between the groups were found (Table 6).

Table 6. Comparisons between control and SFA mothers in movement during the openfield task and the time spent in the center of the openfield area (time in center) in the course of gestation and lactation (only mothers tested) (means ± SE).

Behavior	Stage	p	Mean (± SD)
Movement	Gestation	Z = -2.018, **p = 0.044**	Control: 36.62 (± 60.64)
			SFA: 8.71 (± 11.76)
	Lactation	Z = -0.897, p = 0.37	Control: 20.86 (± 44.21)
			SFA: 13.14 (± 11.94)
Time in center	Gestation	Z = -0.239, p = 0.81	Control: 147.48 (± 216.70)
			SFA: 142.05 (± 204.74)
	Lactation	Z = -0.832, p = 0.405	Control: 54.86 (± 53.77)
			SFA: 77.29 (± 168.37)

Regarding differences between the reproductive stages, control mothers moved more during gestation than during lactation, whereas in the SFA group no difference between the stages was found (control: Z = -2.197, p = 0.028; SFA: Z = -0.845, p = 0.398). The time spent in the center of the openfield area was similar during the reproductive stages in both groups (control: Z = -0.676, p = 0.499; SFA: Z = -0.676, p = 0.499).

When mothers were tested with their offspring in the openfield, no differences in movement, time spent in the center, lactation duration or “side by side” could be detected between the groups ($p > 0.05$).

Cortisol concentrations, both basal and after the openfield test did not differ between the groups (basal: Z = -0.957, p = 0.339; openfield: Z = -1.35, p = 0.177) (Fig. 7). During the openfield test no significant increase in cortisol levels was detected in any of the groups (control: T = -1.980, p = 0.09; SFA: Z = -0.676, p = 0.499) (Fig. 7).

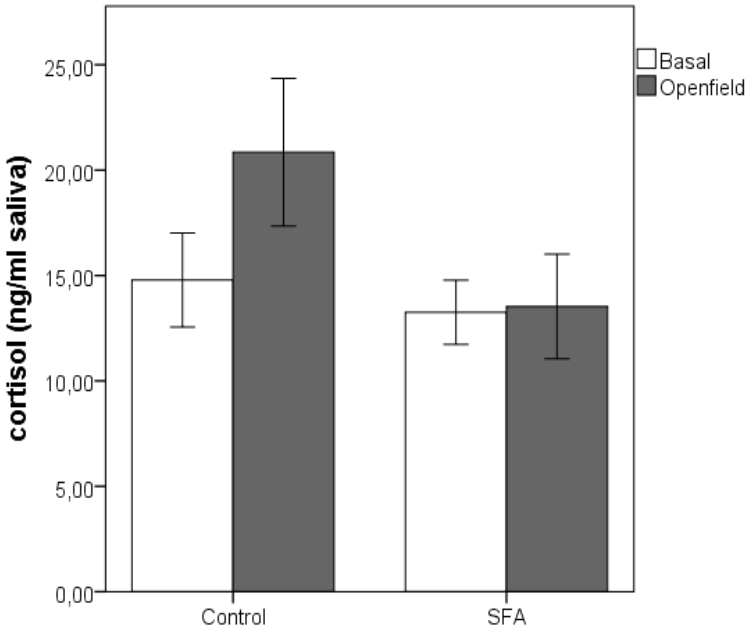

Fig. 7. Cortisol (ng/ml saliva) before and after the openfield task in control (n = 14) and SFA (n = 14) females (phases pooled, means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

Pooled cortisol concentrations (basal and openfield), however, were lower in the SFA group than in controls during lactation (gestation: Z = -0.534, p = 0.593; lactation: Z = -2.12, p = 0.027) (Fig. 8).

Control group showed a significant increase of pooled cortisol values from gestation to lactation, while in SFA females no difference between the stages was found (control: Z = -2.201, p = 0.028; SFA: Z = -.0178, p = 0.859) (Fig. 8).

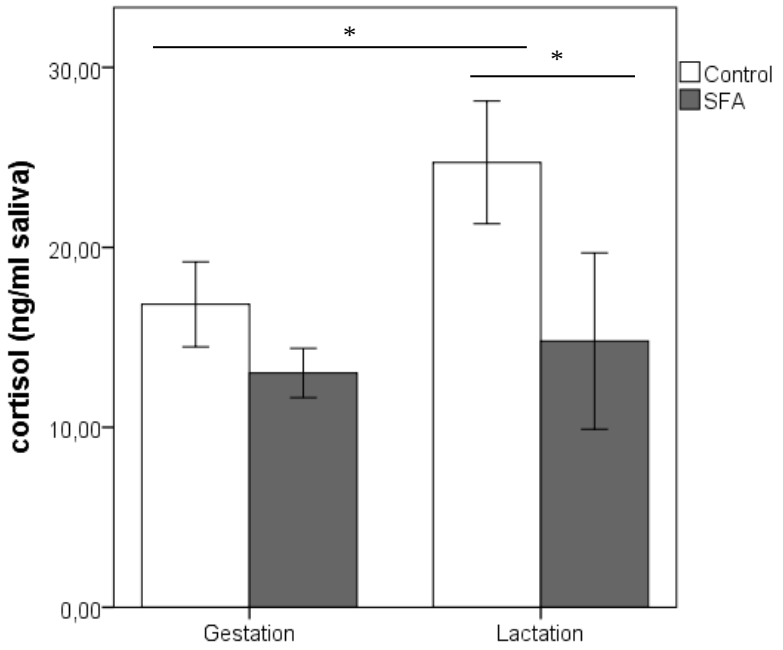

Fig. 8. Pooled saliva cortisol concentrations (basal + openfield) during gestation and lactation in control (n = 10) and SFA (n = 13) females (means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

Juvenile development

Until weaning (first 20 days) SFA offspring gained significantly more body mass (%) than juveniles of the control group (control 11.9 g ± 1.7; SFA 13.2 g ± 2.1; Z = -1.930, p = 0.05). Female offspring in the SFA group gained more mass than female pups of the control group, while no such differences were found for male juveniles (females: Z = -1.952, p = 0.051; males: $F_{0.675}$ = 0.429, p = 0.688) (Fig. 9). Within the groups, however, no sex differences were detected (control: $F_{1.248}$ = 0.288, p = 0.575; SFA: Z = -0.651, p = 0.515) (Fig 9).

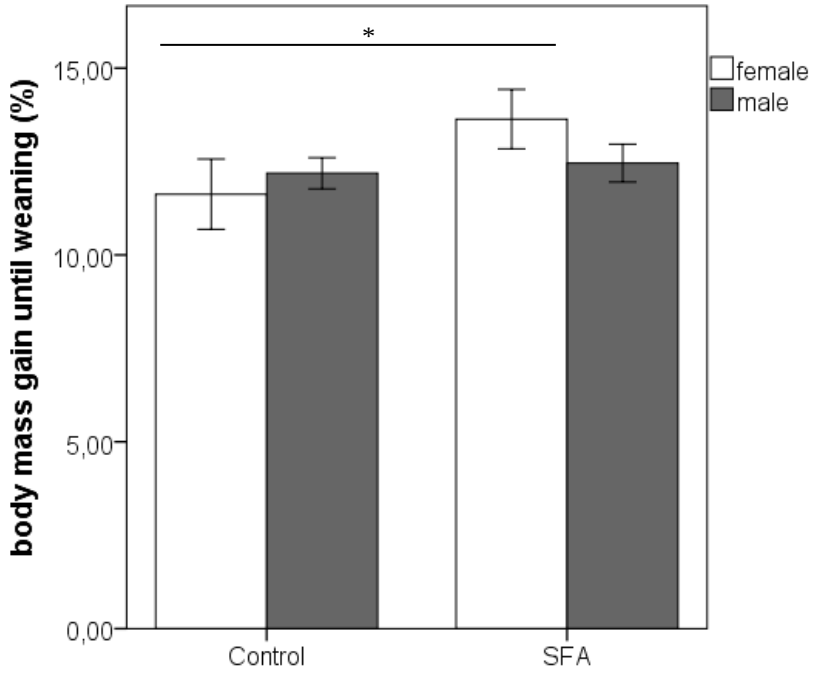

Fig. 9. Body mass gain (%) in juvenile females and males of both groups until weaning: control (n = 13), SFA (n = 16) (means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

In the first 20 days postweaning the relative body mass gain did not differ between control and SFA offspring (Z = -1.666, p = 0.096). Neither female nor male SFA offspring, gained more mass than control females or males (females: $F_{2.687}$ = 0.123, p = 0.098; males: Z = -0.143, p = 0.886). SFA male offspring, however, gained more body mass than females, while no sex differences could be detected in the control group (control females: 34.3 g ± 1.0, control males: 35.0 g ± 1.7, Z = -0.429, p = 0.668; SFA females:31.8 g ± 3.3, SFA: males 34.9 g ± 1.9, $F_{0.859}$ = 0.370, p = 0.05).

When juveniles were tested without their mothers in the openfield, SFA juveniles moved less than controls ($F_{0.771}$ = 0.388, p = 0.027) (Fig. 10).

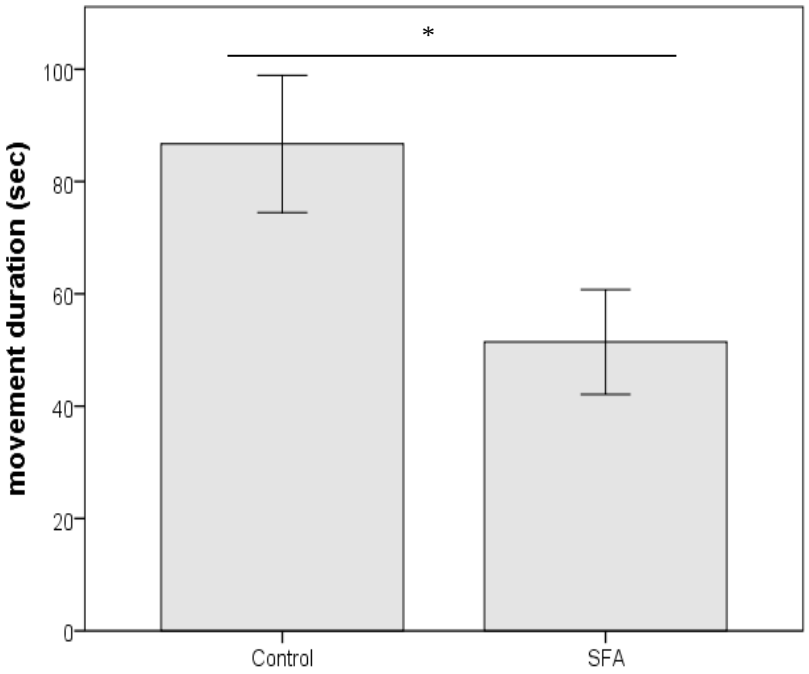

Fig. 10. Movement duration (sec) of the control and SFA offspring in the openfield area (means ± SE). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

Furthermore, SFA female offspring moved less than the control female offspring (Table 7).

Table 7. Comparisons of movement in male and female control and SFA juveniles during the openfield task (only juveniles tested) (means ± SE).

Behavior	Group/Sex	p	Mean (± SD)
Movement	Control	$F_{15.676} = 0.002$, p = 0.279	Females: 101.0 (± 23.75)
			Males: 74.43 (± 55.14)
	SFA	$F_{2.112} = 0.168$, p = 0.243	Females: 60.1 (± 39.03)
			Males: 37.0 (± 32.14)
	Females	$F_{6.081} = 0.027$, **p = 0.021**	Control: 101.0 (± 23.75)
			SFA: 60.1 (± 39.03)
	Males	$F_{7.127} = 0.022$, p = 0.16	Control: 74.43 (± 55.14)
			SFA: 37.0 (± 32.14)

In the openfield test with their mothers, SFA juveniles moved significantly less than the offspring of the control group (Table 8). Other differences between the groups could not be detected in any of the observed behaviors (Table 8).

Table 8. Comparisons of movement, time spent in the center of the openfield area (time in center), suckling and "side by side" in control and SFA juveniles during the openfield task (offspring with mothers tested) (means ± SE).

Behavior	p	Mean (± SD)
Movement	Z = -2.259, **p = 0.024**	Control: 67.46 (± 57.64)
		SFA: 27.73 (± 19.04)
Time in center	Z = -0.624, p = 0.532	Control: 32.54 (± 48.31)
		SFA: 17.87 (± 18.22)
Suckling	Z = -1.267, p = 0.205	Control: 180.77 (± 155.72)
		SFA: 281.67 (± 212.33)
"Side by side"	Z = -0.599, p = 0.549	Control: 302.31 (± 115.91)
		SFA: 272.87 (± 224.24)

Additionally, SFA males suckled more and showed less "side by side" with their mothers than control males (Table 9).

Table 9. Comparisons of suckling and "side by side" in male and female control and SFA juveniles during the openfield task (offspring with mothers tested) (means ± SE).

Behavior	Group/Sex	p	Mean (± SD)
Suckling	Females	Z = -0.354, p = 0.724	Control: 179.83 (± 194.81)
			SFA: 209.56 (± 201.18)
	Males	Z = -2.00, **p = 0.046**	Control: 181.57 (± 129.89)
			SFA: 389.83 (± 195.12)
"Side by side"	Females	Z = -0.707, p = 0.48	Control: 287.17 (± 142.75)
			SFA: 337.44 (± 223.40)
	Males	Z = -2.00, **p = 0.046**	Control: 315.29 (± 97.30)
			SFA: 176.0 (± 205.36)

Within group comparisons revealed no differences between male and female pups in suckling (control: $F_{0.473}$ = 0.506, p = 0.985; SFA: Z = -1.650, p = 0.099) and "side by side" (control: $F_{0.678}$ = 0.428, p = 0.682; SFA: Z = -1.296, p = 0.195).

DISCUSSION

The aim of this study was to determine the effects of saturated and unsaturated fatty acids on the course of gestation and lactation in domestic guinea pigs (*Cavia f. porcellus*). Saturated fatty acids did not affect litter size, offspring sex ratio or the number of alive/dead born pups. In contrast to these results, other studies revealed that saturated fatty acids affected reproductive success, like increased body mass at birth, shifting the sex ratio towards either males (with ad libitium high saturated fat diet) or females (with restricted high saturated fat diet) or lowering the litter size (Alexenko et al. 2007; Bilbo & Tsang 2010; Nemeth et al. 2017). Interestingly, all pups in the UFA group were born alive and litter sizes were highest. These findings concur with previous studies, claiming that diets high in polyunsaturated fatty acids can improve reproductive success and diminish pregnancy loss (Ambrose et al. 2006; Wathes et al. 2007). However, as only two females in the UFA group became pregnant, effects of fatty acids on gestation and lactation could not be tested in this study.

Regarding body mass during the reproductive phases, SFA females were heavier during gestation than during lactation and also had a higher body mass than the control group in both stages. Although both groups had unlimited access to food during pregnancy and lactation, the diet high in saturated fatty acids significantly affected body mass in the SFA group. Since saturated fatty acids are accumulated as body fat with a low rate of β-oxidation and are less preferentially mobilized, the additional fatty acids in the SFA group enabled them to accumulate more fat reserves (Shimomura et al. 1990; Sanz et al. 2000; Raclot & Groscolas 1995).

Adipose tissues are an important source for estrogen production (Nelson & Bulun 2001). During pregnancy, the SFA group differed significantly from the control group in higher estradiol and lower progesterone concentrations. While estrogens plays an important role in the female reproductive cycle, the maintenance of pregnancy and the development of the embryo, progesterone stimulates and maintains indirectly implantation, placentation, placental and fetal development (Lubahn et al. 1993; Bondesson et al. 2015, Bazer et al. 1994). In line with this reverse hormone concentrations, the SFA group had a lower progesterone/estradiol ratio during pregnancy. Progesterone can work as antagonist to estrogens in many reproductive functions by decreasing the quantity of estrogen receptors, hence, higher estradiol concentrations can result in lower progesterone levels (Hsueh et al. 1975). Due to the

accumulation of body fat via saturated fatty acids and the production of estrogens in adipose tissues, higher estradiol concentrations in SFA females could be expected.

Regarding the behavioral aspects, only a few differences could be determined in this study. Whereas sociopositive and agonistic behavior did not differ between the groups, SFA females showed the longest durations of spatial cohesion ("side by side") during gestation, indicating intact social relationships. Moreover, low frequencies of aggressive behavior during group-housing in male and female guinea pigs fed with saturated fatty acids was demonstrated in previous studies (Nemeth et al. 2016a). The SFA group also moved more during lactation than during gestation in the group-housing situation, perhaps because more juveniles wanted to be nursed. One reason for this could be that SFA females lactated not only their own, but also the offspring from other mothers. On the contrary, no difference in movement duration between the stages was found in the control group.

In the openfield test, the SFA group moved less during pregnancy than the control group. In this task, guinea pigs were separated from their social group, to test locomotor activity and explorative behavior. Isolating guinea pigs can be a strong stressor, affecting the HPA-axis and causing temporary immobility ("freezing", Hennessy & Ritchey 1987; Bayard 1957; Miller & Murray 1966). Since cortisol levels did not rise after the openfield test, it can be assumed that the lower activity reflected in movement duration was not related to freezing behavior in response to a stressor but was caused by resting. Additionally, no difference between the groups in the time spent in the center of the openfield area was detected. This would support the idea that the SFA mothers were relaxed, because anxious/stressed guinea pigs would have shown less explorative behavior and would have spent less time in the center of the openfield compared to the control group.

SFA pups moved less in the openfield test than control ones. As we did not take saliva samples from juveniles, cortisol comparisons between the juvenile groups were not possible. However, as it seemed like the SFA mothers showed more resting behavior during the openfield task and had constant cortisol levels, it is very likely that this was reflected in the offspring.

Cortisol concentrations of the females did not increase the openfield task in both groups, which could be explained by the ability of pregnant and lactating mothers to buffer glucocorticoid levels (Reeder & Kramer 2005). In laboratory rats and other mammals, stress-induced cortisol levels are decreased during gestation and lactation, because of increased corticosteroid-binding capacity (Reeder & Kramer 2005). High levels of glucocorticoids in pregnant and lactating

mothers can affect developmental processes in the fetus or in the neonate by entering via the placenta or maternal milk (Welberg & Seckl 2001; Reeder & Kramer 2005). Hence, mothers need to minimize large fluctuations of glucocorticoids. This leads to the assumption that either the mothers were able to buffer the stress of being isolated, or isolation simply did not cause stress for the animals. Nevertheless, former studies in our department with the guinea pigs showed that a temporary isolation did not cause stress, therefore, we assume that the equal cortisol concentrations before and after the openfield test occurred because they were able to relax and avoid social confrontations (Machatschke et al. 2011; Nemeth et al. 2015).

Due to the fact that cortisol levels in the openfield test did not differ from basal levels, group-housing cortisol concentrations and openfield cortisol concentrations were pooled for further comparisons. While suckling increases ACTH and corticosterone release, and glucocorticoids are important for the maintenance of lactation, SFA females did not show any increase of pooled cortisol during lactation, in contrast to the control females (Voogt et al. 1969; Cowie & Tindal 1971). Thus, nursing did not lead to elevated cortisol levels in the SFA group, whereas the control group had significantly higher pooled cortisol values. Regarding effects of saturated fatty acids on cortisol values, only little research has been done yet. Nemeth and colleagues showed that saturated fatty acids elevated saliva cortisol concentrations in male and female guinea pigs during group-housing, however, in females at conception no effects of the diet on glucocorticoids were found (Nemeth et al. 2016a; Nemeth et al. 2017).

The lower pooled cortisol concentrations during lactation in the SFA females indicate lower stress levels, which is interesting because in contrast to the control group, the SFA group behaved more tolerant towards the offspring of other females in their group and lactated them. Allolactation, the nursing of offspring which is not their own, is a phenomenon known in several mammalian species (MacLeod & Clutton-Brock 2015; Packer et al. 1992; Roulin 2002). Gaining mutual benefits, like increasing the indirect fitness of the helpers, reciprocity, improving their maternal skills, reducing the chance to be ejected from the group or mistaken parental care, are the most discussed explanations for allonursing in cooperative breeding vertebrates (Packer et al. 1992; Pusey & Packer 1994; Creel et al. 1991; Wilkinson & Baker 1988; König 1997; Clutton-Brock 2002).

In the SFA group, mothers lactated their own and other offspring equally long. Moreover, the rejection rates of the mothers did not differ between their own and other infants and the suckling attempts were initiated by the offspring. Therefore, it is most likely that allolactation

occurred because the SFA pups tried to get milk from their own, as well as from other mothers. Besides this, SFA mothers could be more tolerant towards the pups because they were able to afford lactating several pups energetically more easily, due to the supplementation with saturated fatty acids. The control females lactated non-offspring only for a few seconds and it seemed that the mothers did not immediately notice that pups of other females had approached.

Milk contains proteins, fats, lactose, minerals and vitamins to ensure the development of the neonate (Jensen 1995). In cows, saturated fatty acids maintained or increased the fat content of the milk, whereas unsaturated fatty acids reduced it (MacLeod & Wood 1972; Dhiman et al. 1995). This would support the assumption that the milk of the SFA mothers was more nutritious and enabled them to lactate not only their own but also other pups. It should however be kept in mind that guinea pigs are highly precocial, have a short lactation period and are able to consume solid food in the first days of their life (Künkele & Trillmich 1997). Therefore, they are quite independent from their mothers and would be able to survive without lactation. As a consequence, mothers from precocial offspring may not be selective in nursing as females raising altricial offspring. The diet of the SFA mothers might have enabled them to act energetically more generously towards all pups in the group.

The fact that SFA offspring, especially females, gained significantly more body mass than the control pups until weaning, also indicated that the SFA young were lactated more often and/or that the higher fat content in the milk of the SFA mothers delivered more energy to the pups. After weaning, when the offspring had no longer access to mother's milk, no difference in the relative body mass gain could be detected between the groups. Precocial newborns have high maintenance costs after birth, because they are active, large, endothermic and highly mobile, resulting in a negative energy balance and a weight loss of about 5 % in the first days after birth (Künkele & Trillmich 1997; Raffel et al. 1996). This mass loss is mainly due to fat reduction, thus additional fatty acids in the mother's milk could counteract the negative energy balance (Raffel et al. 1996; Widdowson 1950). Moreover, newborn guinea pigs have about 10 % body fat, which is also required for thermoregulation in highly developed, precocial mammals (Widdowson 1950; Brück 1967). In contrast, altricial mammals like rats or mice are born with 1-2 % body fat (Widdowson 1950). Therefore, it is likely that SFA pups had a higher body fat content at birth than the control group, and were able to use this additional energy for growth besides thermoregulation and muscle activity.

During lactation, SFA mothers had a higher body mass than the control mothers, indicating that potential costs of allolactation could be compensated by the diet. In general, the efficiency of energy transformation from the mothers to the offspring in guinea pigs during gestation is twice as high compared to lactation (Künkele 2000). Künkele also showed that energy costs during pregnancy are lower than during lactation in guinea pigs, and that the average daily energy intake during lactation is much higher than during gestation (Künkele 2000). Furthermore, energy acquisition and expenditure peaks during early lactation, indicating that lactation is more costly than gestation, even for precocial animals (Künkele & Trillmich 1997).

Maternal energy expenditure can be measured via either food intake or mass loss of the mothers (Gordon 1996). In our study, mass loss did not differ between the control and SFA group, while food intake could not be measured. During lactation, the precocial European hares (*Lepus europaeus*) assimilated high amounts of energy with low amounts of high-fat food (Hackländer et al. 2002). This massive energy yield from high-fat food cannot be reached with low quality food, even with increased food intake (Hackländer 2002). Additionally, high-fat diets can improve milk production and quality (Hackländer et al. 2002). Therefore, feeding additional fatty acids could supply the guinea pigs with extra energy to produce more milk, improve the quality of the milk and lower the costs for the mothers during lactation. This enabled females to lactate offspring from other mothers, while benefits for the pups were obvious in increased body mass gain until weaning.

To summarize the results, saturated fatty acids appeared to have positive effects on female guinea pigs as reflected in higher body mass, more cuddling behavior with their conspecifics, allolactation and lower cortisol values during lactation. As a consequence, we assume that the effects of saturated fatty acids are due to a higher body fat deposition, resulting in a better milk quality, allolactation and favoring the growth of the offspring. Our study revealed that saturated fatty acids can have positive impacts on energy balance, physiology and behavior in pregnant and lactating females, however, further investigations are needed to determine the underlying mechanisms.

References

Abbey M., Noakes M., Belling G. B., Nestel P. J. (1994) Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. The American Society for Clinical Nutrition 59 (5) 995 – 999

Alexenko A.P., Mao J., Ellersieck M.R., Davis A.M., Whyte J.J., Rosenfeld C.S., Roberts R.M. (2007) The contrasting effects of ad libitum and restricted feeding of a diet very high in saturated fats on sex ratio and metabolic hormones in mice. Biology of Reproduction 77 (4): 599 – 604

Altman J. (1974) Observational study of behavior: sampling methods. Behavior 49 (3): 227-267

Ambrose D. J., Kastelic J.P., Corbett R., Pitney P.A., Petit H.V., Small J.A., Zalkovic P. (2006) Lower Pregnancy Losses in Lactating Dairy Cows Fed a Diet Enriched in α-Linolenic Acid. Journal of Dairy Science 89 (8): 3066 – 3074

Bauer B., Womastek I., Dittami J., Huber S. (2008) The effects of early environmental conditions on the reproductive and somatic development of juvenile guinea pigs (Cavia aperea f. porcellus). General and Comparative Endocrinology 155 (3): 680 – 685

Bayard J. (1957) The duration of tonic immobility in guinea pigs. Journal of Comparative and Physiological Psychology 52 (2): 130 – 133

Bazer F. W., Ott T. L., Spencer T. E. (1994) Pregnancy recognition in ruminants, pigs and horses: Signals from the trophoblast. Theriogenology 41 (1): 79 – 94

Bilbo S. D., Tsang V. (2010) Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. The FASEB Journal 24 (6): 2104 – 2115

Bondesson M., Hao R., Lin C. Y., Williams C., Gustafsson J. A. (2015) Estrogen receptor signaling during vertebrate development. Biochimica et Biophysica Acta 1849 (2): 142 – 151

Bourre J. M., Francois M., Youyou A. Dumont O., Piciotti M., Pascal G., Durand G. (1989) The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning task in rat. Journal of nutrition 119 (1): 1880 – 1892

Brück K. (1967) Die Bedeutung des braunen Fettgewebes für die Temperaturregelung des neugeborenen und kälteadaptierten Säugers. Naturwissenschaften 54 (7): 156 – 162

Buydens-Branchey L., Branchey M., Hibbeln J. R. (2008) Associations between increases in plasma n-3 polyunsaturated fatty acids following supplementation and decreases in anger and anxiety in substance abusers. Progress in Neuro-Psychopharmacology and Biological Psychiatry 32 (2): 568 – 575

Campoy C., Escolano-Margit M. V., Anjos T., Szajewska H., Uauy R. (2012) Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. The British journal of nutrition 107 (Suppl. 2): 85 – 106

Clutton-Brock T. (2002): Breeding together: kin selection and mutualism in cooperative vertebrates. Science 296 (5565): 69 – 72

Colangelo L. A., He K., Whooley M. A., Daviglus M. L., Liu K. (2009) Higher dietary intake of long-chain omega-3 polyunsaturated fatty acids is inversely associated with depressive symptoms in women. Nutrition 25 (10): 1011 – 1019

Contreras C. M., Rodriguez-Landa J. F., Garcia-Rios R. I., Cueto-Escobedo J., Guillen-Ruiz G., Bernal-Morales B. (2014) Myristic Acid Produces Anxiolytic-Like Effects in Wistar Rats in the Elevated Plus Maze. BioMed Research International 2014, doi: 10.1155/2014/492141

Cowie A. T., Tindal J. S. (1971) The physiology of Lactation. Experimental Physiology 57 (2): 239 – 240

Creel S. R., Montfort S. L., Wildt D. E., Waser P. M. (1991) Spontaneous lactation is an adaptive result of pseudopregnancy. Nature 351 (6328): 660 – 662

Dhiman T. R., Zanten K. V., Satter L. D. (1995) Effect of Dietary Fat Source on Fatty Acid Composition of Cow's Milk. Journal of the Science of Food and Agriculture 69 (1): 101 – 107

Du Bois T. M., Deng C., Huang X. F. (2005) Membrane phospholipid composition, alterations in neurotransmitter systems and schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 29 (6): 878 – 88

Fedorova I., Salem N. Jr. (2006) Omega-3 fatty acids and rodent behavior. Prostaglandins, Leukotrienes and Essential Fatty Acids 75 (4-5): 271 – 289

Fenton W.S., Hibbeln J., Knable M. (2000) Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. Biological psychiatry 47 (1): 8 – 21

Ferraz A. C., Delattre A. M., Almendra R. G., Sonagli M., Borges C., Araujo P., Andersen M. L., Tufik S., Lima M. M. S. (2011) Chronic omega-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol. Behavioural Brain Research 219 (1): 116 – 122

Gordon L. R. (1996) Trade-offs in energy allocation during lactation. American Zoologist 36 (2): 197 – 204

Hackländer K., Tataruch F., Ruf T. (2002) The Effect of Dietary Fat Content on Lactation Energetics in the European Hare (Lepus europaeus). Physiological and Biochemical Zoology 75 (1): 19 – 28

Hennessy M. B., Ritchey R. L. (1987) Hormonal and behavioral attachment responses in infant guinea pigs. Developmental Psychobiology 20 (6): 613 – 625

Hsueh A. J. W., Peck E. J., Clark J. H. (1975) Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth. Nature 254 (5498): 337 – 339

Joshi H. S., Watson D. J., Labhsetwar A. P. (1973) Ovarian secretion of oestradiol, oestrone, 20-dihydroprogesterone and progesterone during the oestrus cycle of the guinea-pig. Journals of Reproduction and Fertility 35 (1): 177 – 181

Kalmijn S., Launer L. J., Ott A., Witteman J. C., Hofman A., Breteler M. M. (1997) Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Annals of neurology 42 (5): 776 – 782

Kalmijn S. (2000) Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. The journal of nutrition, health and aging 4 (4): 202 – 207

Klausner R. D., Kleinfeld A. M., Hoover R. L., Karnovsky M. J. (1980) Lipid Domains in Membranes. The journal of biological chemistry 255 (4): 1286 – 1295

König B. (1997) Cooperative care of young in mammals. Naturwissenschaften 84 (3): 95 – 104

Künkele J., Trillmich F. (1997) Are Precocial Young Cheaper? Lactation Energetics in the Guinea Pig. Physiological Zoology 70 (5): 589 – 596

Künkele J. (2000) Energetics of gestation relative to lactation in a precocial rodent, the guinea pig (Cavia porcellus). Journal of Zoology 250: 533 – 539

Künzl C., Sachser N. (1999) The behavioral endocrinology of domestication: A comparison between the domestic guinea pig (Cavia aperea f. porcellus) and its wild ancestor, the cavy (Cavia aperea). Hormones and Behavior 35 (1): 28 – 37

Jensen R. G. (1995) Handbook of Milk Composition. London. Academic Press.

Laurien-Kehnen C., Trillmich F. (2004) Maternal food restriction delays weaning in the guinea pig, Cavia porcellus. Animal Behaviour 68 (2): 303 – 312

Lubahn D. B., Moyer J. S., Golding T. S., Couse J. F., Korach K. S., Smiethies O. (1993) Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proceedings of the National Academy of Sciences of the United States of America 90 (23): 11162 – 11166

Lunn J., Theobald H. E. (2006) The health effects of dietary unsaturated fatty acids. British Nutrition Foundation 31 (3): 178 – 224

Machatschke I. H., Wallner B., Schams D., Dittami J. (2004) Social environment affects peripheral oxytocin and cortisol during stress responses in guinea-pigs. Ethology 110: 161 – 176

Machatschke I. H., Bauer B., Glenk L. M., Millesi E., Wallner B. (2011) Spatial learning and memory differs between single and cohabitated guinea pigs. Physiology & Behavior 102 (3-4): 311 – 316

MacLeod K. J., Clutton-Brock T. H. (2015) Low costs of allonursing in meerkats: mitigation by behavioral change? Behavioral Ecology 26 (3): 697 – 705

MacLeod G. K., Wood A. S. (1972) Influence of Amount and Degree of Saturation of Dietary Fat on Yield and Quality of Milk. Journal of Dairy Science 55 (4): 439 – 445

Mattos R., Staples C. R., Thatcher W. W. (2000) Effects of dietary fatty acids on reproduction in ruminants. Journals of Reproduction and Fertility 5 (1): 38 – 45

Miller J. D., Murray F. S. (1966) Guinea pig's immobility response to sound: Threshold and habituation. Journal of Comparative and Physiological Psychology 61 (2): 227 – 233

Moon M. L., Joesting J. J., Lawson M. A., Chiu G. S., Blevins N. A., Kwakwa K. A., Freund G. G. (2014) The saturated fatty acid, palmitic acid, induces anxiety-like behavior in mice. Metabolism clinical and experimental 63 (9): 1131 – 1140

Morris M. C., Evans D. A., Bienias J. L., Tangney C. C., Bennett D. A., Wilson R. S., Aggarwal N., Schneider J. (2003a) Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Archives of Neurology 60 (7): 940 – 946

Morris M. C., Evans D. A., Bienias J. L., Tangney C. C., Bennett D. A., Aggarwal N., Schneider J., Wilson R. S. (2003b) Dietary fats and the risk of incident Alzheimer disease. Archives of Neurology 60 (2): 194 – 200

Nemeth M., Millesi E., Wagner K. H., Wallner B. (2015) Sex-Specific Effects of Diets High in Unsaturated Fatty Acids on Spatial Learning and Memory in Guinea Pigs. Plos One 10: 10, doi: 10.1371/journal.pone.0140485

Nemeth M., Millesi E., Puehringer-Sturmayr V., Kaplan A., Wagner K. H., Quint R., Wallner B. (2016a) Sex-specific effects of dietary fatty acids on saliva cortisol and social behavior in guinea pigs under different social environmental conditions. Biology of Sex Differences 7: 51, doi: 10.1186/s13293-016-0107-5

Nemeth M., Pschernig E., Wallner B., Millesi E. (2016b) Non-invasive cortisol measurements as indicators of physiological stress responses in guinea pigs. PeerJ 18: 4, doi: 10.7717/peerj.1590

Nemeth M., Millesi E. , Siutz C., Wagner K. H., Quint R., Wallner B. (2017) Reproductive performance and gestational effort in relation to dietary fatty acids in guinea pigs. Journal of Animal Science and Biotechnology 8: 28, doi: 10.1186/s40104-017-0158-4

Nettleton J. A., Katz R. (2005) n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: A review. Journal of the American dietetic association 105 (3): 428 – 440

Nelson L. R., Bulun S. E (2001) Estrogen production and action. American Academy of Dermatology 45 (3): 116 – 124

Packer C., Lewis S., Pusey A. (1992) A comparative analysis of non-offspring nursing. Animal Behaviour 43 (2): 265 – 281

Palme R., Möstl E. (1993) Biotin-streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar faeces. In: Görög S, editor. Proceedings of the 5th Symposium on the Analysis of Steroids. Akademiai Kiado: 111 – 117

Palme R., Möstl E. (1997) Measurements of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Zeitschrift für Säugetierkunde 62: 192-197

Pietinen P., Ascherio A., Korhonen P., Hartman A. M., Willet W. C., Albanes D., Virtamo J. (1997) Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. The American Journal of Epidemiology 145 (10): 876 – 887

Pusey, A. E., Packer, C. (1994) Non-offspring nursing in social carnivores: minimizing the costs. Behavioral Ecology 5 (4): 362 – 374

Raclot T., Groscolas R. (1995) Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. Journal of Lipid Research 36 (10): 2164 – 2173

Raffel M., Trillmich F., Houner A. (1996) Energy allocation in reproducing and non-reproducing guinea pig (Cavia porcellus) females and young under ad libitum conditions. Journal of Zoology 239 (3): 437 – 452

Reeder D. M., Kramer K. M. (2005) Stress in Free-Ranging Mammals: Integrating Physiology, Ecology, and Natural History. Journal of Mammalogy 86 (2): 225 – 235

Rood J. (1972) Ecological and behavioural comparisons of three genera of Argentine cavies. Animal Behaviour Monographs 5 (1): 1 – 83

Roulin A. (2002) Why do lactating female nurse alien offspring? A review of hypotheses and empirical evidence. Animal Behaviour 63 (2): 201 – 208

Rowlands I. W. (1949) Post-partum breeding in the guinea-pig. The Journal of Hygiene 47 (3): 281 – 287

Rustan A. C., Drevon C. A. (2005) Fatty acids: Structures and Properties. Encyclopedia of life sciences, doi: 10.1038/npg.els.0003894

Sachser N. (1994) Sozialphysiologische Untersuchungen an Hausmeerschweinchen. Gruppenstrukturen, soziale Situation und Endokrinium, Wohlergehen. Berlin. Paul Parey Verlag: 21–23

Sanz M., Lopez-Bote C. J., Menoyo D., Bautista J. M. (2000) Abdominal fat deposition and fatty acid synthesis are lower and beta-oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. The Journal of Nutrition 130 (12): 3034 – 3037

Sapolsky R. M., Romero L. M., Munck A. U. (2000) How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. Endocrine Reviews 21 (1): 55 – 89

Selle R.M. (1922) Changes in the vaginal epithelium of the guinea-pig during the oestrous cycle. American journal of Anatomy 30 (4): 429 –449

Shimomura Y., Tamura T., Suzuki M. (1990) Less Body Fat Accumulation in Rats Fed a Safflower Oil Diet Than in Rats Fed a Beef Tallow Diet. The Journal of Nutrition 120 (11): 1291 – 1296

Simpoulos A. P., Leaf A., Salem N (1999) Workshop on the Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids. Journal of the American College of Nutrition 43 (2): 127 – 130

Sinn N., Bryan J., Wilson C. (2008) Cognitive effects of polyunsaturated fatty acids in children with attention deficit hyperactivity disorder symptoms: a randomized controlled trial. Prostaglandins, Leukotrienes and Essential Fatty Acids 78 (4 – 5): 311 – 326

Stillwell W., Ehringer W., Jenski, L. J. (1993) Docosahexaenoic acid increases permeability of lipid vesicles and tumor cells. Lipids 28 (2): 103 – 108

Voogt J. M., Sar M., Meites J. (1969) Influence of cycling, pregnancy, labor, and suckling on corticosterone-ACTH levels. American Journal of Physiology 216 (3): 655 – 658

Wang C., Harris W. S., Chung M., Lichtenstein A. H., Balk E. M., Kupelnick B., Jordan H. S., Lau J. (2006) The american journal of clinical nutrition 84 (1): 5 – 17

Wathes D. C., Abayasekara D. R. E., Aitken R. J. (2007) Polyunsaturated fatty acids in male and female reproduction. Biology of Reproduction 77 (2): 190 – 201

Welberg L. A., Seckl J. R. (2001) Prenatal Stress, Glucocorticoids and the Programming of the Brain. Journal of Neuroendrocrinology 13 (2): 113 – 128

Widdowson E. M. (1950) Chemical composition of newly born mammals. Nature 166 (4224): 626 – 628

Wilkinson G. S., Baker A. E. M. (1988) Communal nesting among genetically similar house mice. Ethology 77 (2): 103 – 114

Yamamoto N., Hashimoto A., Takemoto Y., Okuyama H., Nomura M., Kitajima R., Togashi T., Tamai Y. (1988) Effect of dietary alpha-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process, and glycolipid composition. Journal of Lipid Research 29 (8): 1013 – 1021

Yehuda S., Rabinovitz S., Mostofsky D.I. (1997) Effects of essential fatty acids preparation (SR-3) on brain biochemistry and on behavioral and cognitive fractions. In Yehuda S, Mostofsky DI, editors. Handbook of essential fatty acids biology: biochemistry, physiology and behavioral neurobiology. Totowa. Humana Press: 427 – 452

Yehuda S., Rabinovitz S., Mostofsky D. I. (1999) Essential fatty acids are mediators of brain biochemistry and cognitive functions. Journal of neuroscience research 56 (6): 565 – 570

Yehuda S., Rabinovitz S., Mostofsky D.I. (2005) Essential fatty acids and the brain: from infancy to aging. Neurobiology of Aging. 26 (Suppl. 1): 98 – 102

Youdim K. A., Martin A., Joseph J. A. (2000) Essential fatty acids and the brain: possible health implications. International journal of developmental neuroscience (4-5): 383 – 399

Young W. C. (1937) The vaginal smear picture, sexual receptivity and the time of ovulation in the guinea pig. Anatomical Record 67 (3): 305 – 325

APPENDIX

Abstract

Pregnancy and lactation are highly energy-demanding periods in the life of mammals. Therefore, sufficient nutrition is required to guarantee the offsprings' pre- and postnatal survival and appropriate development. Fat provides most energy and is thus a convenient source to ensure enough energy supply during gestation and lactation. To investigate the effects of different fatty acids, our model species, pregnant domestic guinea pigs (*cavia f. porcellus*), were supplemented either with coconut fat (high in saturated fatty acids – SFA) or with walnut oil (high in unsaturated fatty acids – UFA). Due to the fact that only two pregnant UFA females were available for this study, they were excluded from statistical analyses. Effects of the diet groups were determined by monitoring body mass, fecal estradiol metabolites, fecal progesterone metabolites, saliva cortisol concentrations, social behavior, performance in openfield tests, reproductive output and juvenile development.

Reproductive output, referring to litter size or sex ratio, did not differ between the SFA group and controls. During gestation and lactation, SFA females had a higher body mass than the control group. In addition, SFA females showed more spatial cohesion with other females during gestation, had lower cortisol concentrations during lactation and lower progesterone/estradiol ratio during pregnancy than the control group. Regarding juvenile development, SFA pups gained significantly more body mass until weaning than the control group. Furthermore, SFA females did not only lactate their own offspring, but also pups of other mothers. This kind of nursing, allolactation, could not be observed in the control group. We assume that saturated fatty acids positively affected body fat accumulation, resulting in higher energy reserves in SFA females compared to controls, favouring offsping growth and compensating the potential costs of allolaction.

Keywords: saturated fatty acids, unsaturated fatty acids, gestation, lactation, allolactation, guinea pigs, cortisol, progesterone, estradiol

Zusammenfassung

Säugetiere benötigen vor allem während der Trächtigkeit und Laktation viel Energie. Daher spielt die Ernährung in diesen Phasen eine große Rolle, um eine entsprechende Entwicklung des Fötus und des Neugeborenen sicher zu stellen. Fett stellt besonders viel Energie zur Verfügung und ist daher eine geeignete Quelle um eine angemessene Energieversorgung während der Trächtigkeit und Laktation zu gewährleisten. Um die Effekte von verschiedenen Fettsäuren zu erforschen, wurde die Nahrung trächtiger bzw. laktierender Meerschweinchen (*cavia f. porcellus*) und deren Nachkommen entweder mit Kokosnussöl (reich an gesättigten Fettsäuren – SFA) oder mit Walnussöl (reich an ungesättigten Fettsäuren – UFA) angereichert. Die Effekte dieser Fettsäuren wurden durch Vergleiche von Körpergewicht, Fortpflanzungserfolg und Jungtierentwicklung zwischen den Diätgruppen und Kontrolltieren, die nur Standardpellets ohne Fettanreicherung erhielten, ermittelt. Ebenso wurde Sozialverhalten in der Gruppe aufgenommen, sowie Estradiol- und Progesteronmetaboliten, aus Kotproben sowie, Kortisolkonzentrationen aus Speichelproben analysiert und verglichen. In Openfield Tests konnten Einflüsse der Diäten auf potentielle Stressreaktionen durch soziale Isolation bestimmt werden. Da nur zwei Weibchen aus der UFA- Gruppe trächtig wurden, musste die Studie auf Vergleiche zwischen SFA- und Kontrolltieren beschränkt werden.

In Bezug auf Fortpflanzungserfolg, wie zum Beispiel der Wurfgröße oder dem Geschlechterverhältnis, konnten keine Unterschiede zwischen SFA- und Kontrollweibchen gefunden werden. Während der Trächtigkeit und der Laktation hatten die SFA Weibchen ein höheres Körpergewicht als die Kontrollgruppe. Des Weiteren suchten die SFA Weibchen während der Schwangerschaft in der Gruppenhaltung mehr körperliche Nähe zu den anderen Weibchen, hatten niederere Kortisolspiegel während der Laktation und während der Trächtigkeit ein geringeres Progesteron/Östrogen-Verhältnis als die Kontrollgruppe. Die Jungtierentwicklung unterschied sich ebenfalls indem die SFA Junge signifikant mehr Körpergewicht bis zur Entwöhnung zulegten als die Kontrollgruppe. Außerdem säugten die SFA Mütter nicht nur ihre eigenen Jungen, sondern auch die von anderen Müttern. Diese Form des Laktierens, die Allolaktation, konnte in der Kontrollgruppe nicht festgestellt werden. Eine mögliche Erklärung wäre, dass die SFA Mütter aufgrund des höheren Fettkonsums und der dadurch vorhandenen Reserven, energetisch mehr Spielraum hatten und die potentiellen Kosten von Allolaktation durch ihre Diät kompensieren konnten. Der Nachwuchs profitierte von der SFA-Diät durch gesteigerter Gewichtszunahme bis zur Entwöhnung. Zusammenfassend

zeigten die Ergebnisse dieser Studie positive Einflüsse gesättigter Fettsäuren auf die Energiebilanz, Sozialverhalten, Östrogensekretion, Stresskompensation und Milchproduktion bei trächtigen und laktierenden Meerschweinchen.

Schlüsselwörter: gesättigte Fettsäuren, ungesättigte Fettsäuren, Trächtigkeit, Laktation, Allolaktation, Meerschweinchen, Kortisol, Progesteron, Estradiol